mechanisms. Since growing crystals of large complexes that are heterogeneously glycosylated like the HA/SP-D or HA/SP-A complexes is difficult, we have undertaken computational studies to model the interactions. Crystal structures of HA, SP-A, and SP-D with small saccharide ligands offer starting points for modeling full length complex type or high-mannose glycosylation using low energy sugar chain conformations derived from solution studies. Further computational approaches are used to obtain an ensemble of potential complex structures. Analysis of these structures can give insights into the mechanisms of inhibition of SP-D and SP-A variants, as well as the specificity of SP-D and SP-A for certain glycoforms of HA. Measurement of the differences in glycosylation site occupation and the glycans residing on HA and SP-A or SP-D is an important adjunct to the molecular modeling. A complete strategy based on liquid chromatography/tandem mass spectrometry analysis is underway to investigate the different glycosite and glycoforms present.

1173-Pos Board B83

Small Molecule HIV-1 Capsid Inhibitor Design using Hybrid Structure Based Methods

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Protein-protein interactions (PPI) are fundamental to almost all biological and pathological processes and therefore represent an important class of therapeutic targets that can be utilized against multi-drug resistant pathogens such as HIV-1. The HIV-1 capsid (CA) protein has recently emerged as an attractive target as it performs essential roles, both regulatory and structural in early and late stages of the viral life cycle. Hence, small-molecule inhibitors of capsid assembly would be attractive and novel antiretroviral medications. With the latest crystal structure of the hexameric arrangement of CA monomers, atomic level details of the CA hexamer interface are now available. The HIV-1 capsid shell is composed of ~250 CA hexamers and 12 CA pentamers which are arranged in the form of a fullerene cone. Crystal structure analysis shows a weak association between the monomers and hence the interfaces formed between the N-terminal (NTD) and C-terminal domains (CTD) seem attractive as drug targets. In this study, we targeted the NTD-NTD interface region as a novel PPI to design inhibitors using the hybrid structure based method. Our preliminary results show that we have identified two compounds (CK026 and CK422) that display significant antiretroviral activity against HIV-1. Importantly, these compounds belong to two distinct inhibitory classes: early-stage inhibitors and late-stage inhibitors. Compound CK026 represents the first CA-targeted small molecule that works by disruption of pre-integration events in HIV-1 replication. Further chemical modification has led to identification of other analogs that retain the antiviral activity with improved drug like properties.

1174-Pos Board B84

Protein Loop Modeling using Distance-Guided Sequential Monte Carlo Method

Ke Tang, Jinfeng Zhang, Jie Liang.

Modeling loop regions is an important task for protein structure prediction. We have developed a new loop construction method through efficient sampling with a loop-specific energy function. Based on a new sequential Monte Carlo sampling strategy called Distance-guided Sequential Monte Carlo (dSMC), our method efficiently generate loop conformations with lower energy. To derive the loop-specific energy function, a decoy-based reference state is used with a large set of loop conformations. Our approach is works well in modeling long loops. The average smallest global RMSD for 11 residue loops generated is about 1.5 A. Our method also addresses the challenging problem of multi-loop modeling. As loops often are in spatial proximity and interact with each other, our approach treats these loops simultaneously and sample multiple loop efficiently. As an example, for the protein ribonuclease A (PDB id: 7RSA) which has 3 loops (7+4+5 residues), the global RMSD for the loops with the lowest energy compared to the known structure is only 0.52 A.

1175-Pos Board B85

Structure Based Inhibition of Mitochondrial Aldehyde Dehydrogenase (ALDH2) Activity

Ann C. Kimble-Hill, Hina Younus, Samy Meroueh, Thomas D. Hurley.

Mitochondrial aldehyde dehydrogenase (ALDH2) is an enzyme that participates in multiple metabolic pathways, including the oxidation of toxic biogenic and environmental aldehydes. Our laboratory is interested in developing novel and selective ALDH2 inhibitors. We recently identified and studied three distinct classes of small molecules based on their ability to inhibit ALDH2 activity (esterase, dehydrogenase, and both). These inhibitors were identified by virtual screen which consisted of three steps: 1) docking 800,000 ChemBridge molecules to the substrate cavity in ALDH2; 2) scoring the receptor-ligand complexes while computing the binding affinity; and 3) ranking the top 1,000 compounds using GlideScore to determine the top 250 compounds for study. From this list, 112 compounds were selected for purchase and screened for inhibitory activity at 50 uM. 19 compounds were selected for further evaluation based on their ability to inhibit hALDH2 propionaldehyde oxidation by more than 60%. IC50 values for these compounds were determined using both the dehydrogenase and esterase assays.

We selected 4 compounds with IC50 values less than 20uM for further kinetic and structural studies: I32, I72, I76, and I78. These inhibitors show a competitive inhibition pattern toward varied NAD+ concentrations and either uncompetitive or noncompetitive inhibition towards varied priopionaldehyde concentrations. Based on their structural similarity and x-ray crystallography structures obtained with these compounds bound to the enzyme, fragments were then chosen for studying the mechanism by which they inhibit ALDH2. The next step is to solve the structures of these fragments bound to ALDH2 and develop SAR data for fragment based compound development that will enhance their specificity for and inhibition of ALDH2. This work was supported by NIH R01-AA18123 and NIH R01-AA18123S1.

1176-Pos Board B86

Role of water and G Protein in modulating agonist affinity in GPCRs

Supriyo Bhattacharya, Michiel Niesen, Alfonso R. Lam, Nagarajan Vaidehi.

Predicting accurate ligand poses and ligand selective receptor conformations are imperative in designing efficacious, functionally specific drugs for G-protein coupled receptors. Comparison of the crystal structures of carazolol (inverse agonist), formoterol(agonist), and agonist with G-protein mimic bound β2 Adrenergic Receptor (β2AR) shows that the agonist stabilizes a slightly different conformation when there is no G-protein bound. The G-protein bound receptor state exhibits a high affinity conformation for the agonist. Using the computational method LITiCon, we have calculated the activation pathways for full, partial and inverse agonists of β2AR, which are in agreement with fluorescence intensity lifetime measurements. MD simulations starting from various conformations along the activation pathway (total ~1.5 μs) show that in the absence of G protein, norepinephrine (agonist) stabilizes an intermediate receptor state that is similar to the formoterol bound intermediate state without the G-protein, in agreement with the recent crystal structure of formoterol bound β2AR. Thus coupling to G protein may be needed for stabilizing the fully active state.

Using Liticon method we have calculated the activation pathway of agonist bound adenosine receptor A2A starting from its inactive state. We found that water plays a important role in the docking of the antagonist as well as the agonist.

We recently predicted the structures of D3 dopamine receptor and CXCR4 chemokine receptor as part of the competition for the assessment of computational methods in GPCR modeling (GPCR Dock 2010). The predicted docked poses of both the ligands are in close agreement with the crystal structures. The details of the methods and comparison with the crystal structures will be presented.

1177-Pos Board B87

Biophysical Approaches to Discovery of Novel Antibacterial Leads

Gautam Sanyal.

This presentation will outline biophysical approaches to discovery of antibacterial lead compounds that are designed to address bacterial resistance to many current antibiotics. There are a number of essential enzymes, in key pathways of pathogenic bacteria, which have not been targeted by existing drugs. The potential bacterial selectivity and spectrum of these single gene targets can be validated using structural tools. In addition, inhibiting clinically exploited and validated bacterial enzymes with a different mechanism (e.g., at a different binding site) than those offered by current drugs can be a powerful approach. Target enzymes that have remained unexploited or those that offer alternative binding sites include cell wall biosynthesis and DNA replication enzymes. In our discovery efforts, biophysical studies including X-ray crystallography and NMR spectroscopy are utilized at all stages of lead generation beginning with target selection, validation, selectivity analysis and probing of target binding sites for druggability. This will be exemplified with comparative structures of target enzymes from different pathways. Hits identified through screening of compound and fragment libraries against these targets are progressed towards leads with the help of biophysical and structural analysis of protein-ligand complexes. Development of effective screening assays for enzyme targets requires a thorough understanding of enzyme mechanism. An example will be given of how water LOGSY measurements by NMR explained the mechanism of a multisubstrate cell wall enzyme. Finally, development of a lead series against

a bacterial DNA replication enzyme beginning with a fragment based lead generation approach will be presented. In this approach, biochemical, biophysical and structural tools were employed to screen fragment libraries against the target enzyme and progress initial hits to drug-like leads. The availability of high-resolution target structure provided valuable chemistry guidance in designing such leads.

1178-Pos Board B88

ROSETTAEPR: An Integrated Tool for Protein Structure Determination From Sparse EPR Data

Stephanie Hirst, Nathan Alexander, Hassane S. Mchaourab, Jens Meiler.

Membrane proteins remain a particular challenge in structural biology. Only about 1.5% of reported tertiary structures and 60 unique membrane protein topologies consisting of more than one transmembrane span are represented in the PDB. However, these proteins make up an estimated 30-40% of the entire proteome, and over half of all therapeutics target this group. Site-directed spin labeling electron paramagnetic resonance (SDSL-EPR) is often used for the structural characterization of proteins that elude other techniques, such as X-ray crystallography and NMR. However, high-resolution structures are difficult to obtain due to uncertainty in the spin label location and sparseness of experimental data. ROSETTAEPR has been designed to improve high-resolution protein structure prediction using sparse SDSL-EPR distance data. The ''motion-on-a-cone'' spin label model is converted into a knowledge-based potential, which was implemented as a scoring term in ROSETTA. We have demonstrated the feasibility of using ROSETTAEPR with soluble proteins by benchmarking the method on T4-lysozyme. ROSETTAEPR increased the fractions of correctly folded models ($RMSD_C < 7.5$Å) and models accurate at medium resolution ($RMSD_C < 3.5$Å) by 25%. After full-atom refinement, ROSETTAEPR yielded a 1.7Å model of T4-lysozyme, thus indicating that atomic detail models can be achieved by combining sparse EPR data with ROSETTA. ROSETTAEPR was also benchmarked on a set of membrane proteins of known structure. If EPR experimental data were not available, simulated data were derived from the existing structures. It was generally observed that *de novo* folding in the presence of EPR restraints enriched the recovery of the proteins' correct topology compared to when folding with no restraints.

1179-Pos Board B89

Incorporating the Effects of pH in Protein-Protein Docking

Krishna Praneeth Kilambi, Jeffrey J. Gray.

Highly charged interfaces often frustrate protein-protein docking methods. Current approaches do not account for changes in the ionization states which may play a crucial role in binding. Predicting the charges on the residues would be the first step towards understanding the dependence of docking on pH. We developed a method to predict the pKa values of the common ionizable residues in proteins (Asp, Glu, Lys, His and Tyr) . It incorporates conformational flexibility through extensive amino-acid side-chain rotamer sampling and is based on the Rosetta energy function with an explicit term to account for the protonation state probabilities of the amino-acids. In approximately 80% of the cases, the method predicts the pKa value of a residue to an accuracy of 1 pH unit from the experimental value and 95% of the time it predicts a value within 2 units of pH. The method is comparable in accuracy to the other published computational pKa prediction methods and is fast enough to be used to dynamically predict and alter the ionization states of the amino-acid side chains during protein-protein docking. We expect the new protocol to lead to improvements in conformational sampling during docking as well as help in the discrimination and ranking of the generated ensemble of tentative protein complexes.

1180-Pos Board B90

Mechanism and Action of Flufirvitide, a Peptide Inhibitor of Influenza Virus Infection

Hussain Badani, Robert F. Garry, Russell B. Wilson, William C. Wimley.

Influenza is an infectious disease typically transmitted through the air. It is responsible for seasonal epidemics affecting millions of people, and sporadic global pandemics. Influenza infection is a membrane fusion-dependant process, occurring in the endosome of the host cell after viral binding and endocytosis. The virus-host membrane fusion process is mediated by hemagglutinin (HA), a viral surface glycoprotein. Studies show that when the virus is subjected to low pH in the endosome, the HA protein partially unfolds and changes conformation, exposing the fusion initiation region (FIR). A 16 amino acid peptide sequence (Flufirvitide) derived from the fusion initiation region of the HA protein has shown effective inhibition of influenza virus infection. It is hypothesized that there is an interaction between the peptide and the FIR which inhibits fusion of the virus to the host cell. Plaque inhibition assays and animal studies show high efficacy of the peptide against the virus. We are currently developing biochemical and biophysical assays to study the interaction between Flufirvitide and HA. Circular Dichroism studies show that the peptide has a random coil conformation at pH 7 and higher. To elucidate the mechanism of fusion inhibition, the interaction between peptide and HA is being investigated with immunodetection, immunoprecipitation, and florescence techniques. Additionally, binding and interaction of the peptide with the intact virus is being studied by using Cryo-electron microscopy.

1181-Pos Board B91

Computational Design of Small Molecules with Druglike Properties

D.S. Dalafave, K.S. Jani.

Many cancers overexpress antiapoptotic proteins, which can lead to poor chemotherapy response. This work reports on computational design of druglike small molecules that could potentially facilitate apoptosis by forming complexes with antiapoptotic proteins. Drugs based on small molecules that target a single protein can lead to drug resistance. On the other hand, a drug that operates too broadly may harm healthy tissue. An alternative is a drug that can bind two or more of the proteins. A single drug would be more economical and lead to fewer side effects than a combination of drugs with each one targeting a single protein. In this work, structures of experimentally known small molecules were used as templates to design new molecules. Common structural features of the experimental molecules were identified. The Osiris Property Explorer program was employed to study how the features influenced molecular druglike properties. Atomic substitutions and structural modifications were done to design new small molecules. Drug-related properties and potential toxicities of the molecules were determined and compared to those of commercial drugs. Molecules with no indicated toxic risks and optimal values of drug-related properties were used for docking studies in the ArgusLab program. Binding energies of stable configurations of the designed molecules and antiapoptotic proteins were calculated. Designed molecules that made the most stable complexes with two or more antiapoptotic proteins were identified. The potential to use the designed molecules in anticancer drug design is discussed.

1182-Pos Board B92

Total Synthesis of Moscatilines and Wedelolactones as Potential Inhibitors of Anti-Metastic Agents in MDA-MB-231 Cells

Yean-Jang Lee, Wen-Shing Tsau, Chia-Fu Cheng, Tsui-Hwa Tseng, Pei-Yun Huang, Shien-Kai Chuang.

Combretastatins, which are an important group of anticancer drugs, were isolated by Pettit et al. from the African tree Combretum caffrum in 1989. Additionally, Liang et al. have reported that ten coumestans were isolated from the roots of *Hedysarum multijugum*, which is a plant in *Hedysarum* Linn. of the family *Leguminosae* used as a folk herbal drug in northwest China. Coumestans comprise a class of naturally occurring products with a variety of biological activities including phytoestrogenic, antibacterial, antifungal, antimyotoxic, and phytoalexine effects. Due to their biological activities, the synthesis of Hedysarimcoumestan **B** is achieved in which the longest linear sequence is only eight steps in 50% overall yield from commercially available phloroglucinol. The key transformations in the synthesis are Stille coupling and DDQ oxidative-cyclization reactions. This synthetic strategy can be applied to give access to the demethylwedelolactone and wedelolactone, which were afforded from α-bromocoumarin in high 55% and 47% yields, respectively. In addition, CA-4 analogues are also synthesized by Pd-catalyzed coupling in overall yield 20~28%. Furthmore, the molecular model was examined the interactions of proteins and ligands as well. Finally, the bioassay results show that the anti-invasion and anti-metastsis activity of wedelolactones in breast cancer are associated with inhibition of signaling pathway and promotion of chromatin remodeling. Camphorataimide B reduced the size and weigth of lung as well as lung colonization of MDA-MB-231 cells.

1183-Pos Board B93

In Silico Approach to Identify Substrates and Inhibitors of Malaria Proteases

Lauren E. Boucher, Jenny Lundqvist, Holly L. Hammond, Jürgen Bosch.

Malaria affects over 500 million people and causes 1.3 million deaths annually. The parasite, *Plasmodium falciparum*, is responsible for the most deadly variant of malaria in humans. Due to evolving resistance to current treatments, it is necessary to develop new drugs targeting the parasite. Potential drug targets are the malaria proteases; several proteases are critical in both the liver and blood stages of the parasite's life cycle, essential for both invasion and egress of cells. Proteases have been effective drug targets for the treatment of several diseases evidenced by the development of HIV protease inhibitors, ACE inhibitors treating hypertension, and anticoagulants treating thrombosis.

In developing new therapies, we are taking a structure-based drug design approach to design inhibitors of proteases key to parasite survival. While several vital proteases have been identified, relatively little else is known and substrates for many proteases have yet to be identified. To identify these substrates, we are using an *in silico* approach to screen for peptides that bind to the protease active